# THE USE OF A PHOTOELECTRIC COLORIMETER FOR DETERMINING SMALL AMOUNTS OF FLUORIDES AND PHOSPHATES

by

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# Thesis

## The Use of a Photoelectric Colorimeter for Determining Small Amounts of Fluorides and Phosphates

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by

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under the direction of Dr. O.J. Walker

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# The Use of a Photoelectric Colorimeter for Determining Small Amounts of Fluorides and Phosphates.

## PART 1

## Introductory Discussion and the Theory of Colorimetry

### Introduction:

The fluorine content of domestic water supplies and food is of prime importance in the investigation of the effect of fluoride on the functions of the human body. It has been established (7,23,24) that dental fluorosis or mottled enamel is associated with the fluorine content of water. The mottling is produced during the period in which the teeth are developing and calcifying in the gums (7). The fluorine content is, therefore, of some physiological importance. It has been shown that water containing over 0.9 p.p.m.# of fluoride causes dental fluorosis, while, on the other hand, it is believed by some that if the fluoride content is less than this amount there is a possibility that the teeth will be susceptible to dental caries (25).

The development of a rapid and accurate method for determining small amounts of fluorides is needed. This method should be applicable to the determination of fluorides in all types of materials such as food, water supplies, and animal and plant tissues. Evidence indicates that dental fluorosis is caused by the amount of fluoride ingested. Evidence (1) also indicates that dental caries can be prevented, at least in part, by fluorides. The analysis of fluoride in bones, tissue, and metabolic wastes is of great importance in studying the nondental effect of traces of fluorine. Hence it cannot be stressed too much that there is a dire need for a rapid, accurate and efficient method for determining small amounts of fluorine.

#p.p.m. refers to parts per million parts.

Note: The words "fluorine" and "fluoride" are used interchangeably.

Since evidence indicates that dental caries may be prevented, at least in part, by fluorides this matter has been the object of scientific investigation. Two methods of supplying the fluoride have been suggested:

(1) increasing fluoride ingestion;

(2) topical application of fluorides.

The increasing of fluoride ingestion by its addition to the water supply is the most applicable to large population groups. In order to control this fluoride ingestion to the optimum amount it becomes necessary to know the amount present in water supplies before the addition of fluoride, in order to calculate the amount of fluoride to be added. Since the fluoride content of streams and rivers varies, numerous of these determinations would be necessary during each year. Therefore, for this chemical control, it is necessary to have a rapid, simple, and accurate method for determining fluorides.

The analysis for fluoride in bones, tissue and metabolic wastes is of great importance in studying the nondental and dental effects of trace quantities of fluorine. Both dental tissue and the skeletal system are very sensitive to fluorine because of a marked tendency of these tissues to retain fluorine. The investigation of the effect of fluorine on these metabolic processes is greatly aided by a knowledge of the fluorine content of urine, sweat, bones, and teeth. This brings out the necessity of a rapid method for determining fluorides in bones, teeth and metabolic wastes.

The recent increase in the use of fluorides in insecticides has also stimulated interest in the determination of fluorides in small quantities in foods. The United States Food and Drug Administration has placed the maximum allowable limit for fluorine content of food at 1.4 p.p.m.

As yet no reaction has been reported in the literature in which a colored substance is formed by the direct interaction of fluoride ion and some reagent. All of the reported colorimetric methods for determining fluoride ion depend upon thebleaching of a colored compound or lake by the action of the fluoride ion. This fading is generally brought about by the formation of stable complex ions from fluoride ion and the metallic constituent of the colored compound or lake. These complexes are colorless.

The methods of fluorine determination may be roughly divided into titration methods (3,6,33,36) and visual colorimetric methods based on the extent of fading of colored compounds or lakes by fluoride ion. Colorimetric methods based on the bleaching effect of fluorides on the lake formed by sodium alizarin sulfonate and a zirconyl salt have been developed by Sanches (2), Elvove (10) and Scott (22). Methods have been devised which depend upon the bleaching by fluoride ion on a colored ferric iron compound (12,14,35). Okuno has used the fading of an aluminum-hematoxylin lake to determine the concentration of fluoride ion (19). The fading of a thorium-sodium alizarin sulfonate lake has also been used (28).

It has been known that sulfates have an effect on the sodium alizarin sulfonate-zirconyl nitrate indicator similar to that of fluorides(21). Sanchis has modified the method used by Thompson and Taylor (30) for the determination of fluorides in sea water so that it is applicable to the determination of fluorides in natural waters. Sanchis has added sulfuric acid to the indicator reagents to minimize the effect of sulfates in natural waters.He found that sulfates do not interfere unless they are present in amounts of 500 p.p.m. over above the amount added as sulfuric acid. Some waters are unusually high in sulfate, however, and the amounts found are often comparable to those added as sulfuric acid. This gives results higher than they should be.

The effect of sulfates on the lake formed by zirconyl nitrate and sodium alizarin sulfonate has received a great deal of attention from Walker and his co-workers Finlay, Gainer and Murphy. Walker and Finlay (34) found that the effect of sulfates is additive, and were able to apply a correction if the amount of sulfate present was known. Application of this correction for sulfates brought the Sanchis results into agreement with those obtained by a modified Armstrong method (3).

In this modified Armstrong method the fluorine is isolated by distillation over sulfuric acid and the amount of fluorine in the distillate is determined by titration with a standard thorium nitrate solution, using sodium alizarin sulfonate as an indicator.

The effect of sulfates on the red zirconyl nitrate-sodium alizarin lake has been investigated by Murphy (18) using a photoelectric colorimeter. They, also, found that effect of sulfates was additive and were able to apply a correction if the amount of sulfate in a sample was determined. The results, they obtained, agreed with the visual method of Walker and Finlay.

It is well known that phosphates interfere with the determination of fluorides in all the reported colorimetric and volumetric method (8). Most natural water supplies do not contain sufficient phosphate to interfere with the colorimetric methods. When one is determining the amount of fluoride in foods and tissues the interference of phosphates becomes very important in obtaining an accurate fluoride determination. Foods and tissues contain a much greater phosphate content than the average water supply. In this research the interference of phosphate on colorimetric methods has been investigated in the hope that the necessary corrections may be applied.

In the determination of fluorides in foods and tissues a preliminary ashing is necessary to remove the bulk of the organic material so that it will not interfere with the colorimetric determination.

The interference of phosphates can be eliminated by distilling with perchloric acid when organic material is absent (36). When organic material is present, either before or after ashing, it has been found necessary to carry out two separate distillations in order to separate phosphate and fluoride. The first distillation is carried out with concentrated sulfuric acid; this distillation is followed by a distillation with perchloric acid (8). The fluorine in the final distillate may be determined by either the thorium nitrate titration method or by one of the colorimetric methods. The above dist~~t~~illations are very time consuming and require special apparatus.

The development of a method, for the determimation of fluorides in small quantities in all types of material, which is less time consuming than the above distillation methods is much needed. Photoelectric colorimetric methods are rapid and accurate. They are particularly suited for the cases where a large number of similar determinations have to be taken. They exclude to a greater extent personal factors and are generally less fatiguing upon the worker. Colorimetric methods are also well suited for the determination of very small amounts of a substance.

It would be very nice if a photoelectric colorimetric method, which did not have the aforementioned objections of interfering ions, could be developed. The photoelectric colorimeter has been used very little in the determination of small amounts of fluorides. Walker and Gainer (32) used an Evelyn type direct reading colorimeter (11) for the determination of fluoride but found it necessary to use an absorption cell of the order of 15 to 20 cm. Murphy (18) used a Lumetron Photoelectric colorimeter for the same purpose. The bleach-

ing effect of fluoride on a zirconyl-sodium alizarin sulfonate lake was used in both cases. They were able to correct for sulfate interference up to 3600 p.p.m. but were unable to correct for the interference caused by phosphate ion.

This is the starting point of the work carried out as described in this report. A thorough investigation of the effect of sulfates and phosphates on photoelectric colorimetric methods is to be undertaken.

## Colorimetry, Methods and Underlying Theory

The term colorimetry, as used here includes measurement or comparison of colors to determine the amount of some constituent of a solution. The constituent to be determined must be colored, react with a suitable reagent to give a color or, as in the case of fluorine, cause a change in color of one of the reagents.

Solutions used in colorimetry exhibit color as a result of reflection or transmission of unabsorbed radiant energy. Comparison of the colored system containing the unknown constituent with a similar system containing a known amount of that constituent is the basis of chemical colorimetry. Instruments used are therefore more correctly called comparators. Five general methods are used to compare colors.

### (a) Standard Series Method

A series of standards is used. The depths of the solutions in the standard and unknown are the same. The unknown is compared visually with each member of the series until a match is found. The concentration of the desired constituent in the unknown is then the same as that in the standard to which it most nearly conforms or is estimated from the standard. In this way the amount of test substance

is obtained without calculation, because, if the volume and color of the unknown and standard are the same their contents of test substance will be identical. Beer's law need not apply and dichromatism can be tolerated.

The standards may be stable natural standards, unstable natural standards made up at frequent intervals, or artificial standards designed to match the color developed from the test substance. The unknown is referred to as the test substance.

(b) The Dilution Method

The standard and sample are placed in graduated tubes of similar diameter, and the darker diluted with the same concentration of reagents as is present in sample and standard. The end point is obtained when sample and standard give a visual match. When this end point is reached each unit of volume of one solution must contain the same amount of test substance as each unit volume of the other and the amount in the unknown is to the amount in the known directly as their volumes. Beer's law need not apply.

The apparatus for comparison by the dilution method consists essentially of a pair of graduated tubes, viewed crosswise, and protected from sidelight.

(c). Absorption Method

This is closely related to the series of standards method. The transmittancy of a series of developed standards is measured, generally with a restricted wave band. A calibration chart is made by plotting transmittancy or percentage transmittancy vs. concentration of the test substance. The developed unknowns are similarly read and the concentration of the unknown is read directly from the chart. Beer's law need not apply to the system; if it does the transmittance curve will be smooth and if $\log_{10}$ of the transmittance is plotted

against concentration the curve will be a straight line.

The absorption method is used for the work done on the determination of fluorides and phosphates in this report. This method is particularly good when a large number of similar determinations are made.

(d) The Balancing Method

The color of the unknown solution is matched with a standard by adjusting the depths of solution. The concentration of the desired constituent in the two solutions must be inversely proportional to the depths of solution. Beer's law must hold for the system.

The simplest form of apparatus for the balancing method consists of two similarly graduated tubes. The sample is placed in one tube and the standard in the other. The two tubes are held over a piece of white paper and standard solution is poured into the standard tube until the color observed through the lengths of the tubes is identical. The Duboscq colorimeter is based on this principle.

(e) The Duplication Method

The sample is made up to a definite volume, and nearly that volume of distilled water placed in a similar container is treated with the same reagents for bringing out the color of the solution, as were used with the sample. Then a standard stock solution of the constituents being determined is added from a buret until, when the level is brought up to the mark, the color in the two tubes match. The unknown then contains the same amount of the constituent as was added to the comparison tube. Beer's law need not hold for the system but reaction must be almost instantaneous.

Extent of Use of Colorimetric Methods

Colorimetric methods sometimes give results in five minutes to

one hour while similar determination by some other method, may take much longer. Colorimetric methods are in general rapid and reasonably accurate. A broad field of usefulness of colorimetry is the determination of impurities or substances easily soluble in water, acid or base. Colorimetric methods are generally very sensitive, usually detecting test substances in the concentration range 0.5 - 10 p.p.m. Some methods are sensitive enough to detect one part per billion.

In general, in the development of a new colorimetric method, only the methods of a series of standards or absorption can be assumed to be applicable. The standards should contain the same amount of the reagents as the unknown and prepared at the same time.

If the color develops over a wide range of wave bands, then the eye with reasonable accuracy over the entire range will respond to the intensity with greater accuracy than an instrument reading in a restricted wave band. But if the color developed by the reaction of the test substance and reagents lies almost entirely within a restricted waveband, the use of filter methods will be preferred.

Sometimes the color substance obtained from the unknown material precipitates above a certain concentration. This sets an upper limit in some cases. The lower limit is usually that at which the color is just noticeable. The accuracy generally becomes less as the lower limit is approached. Sometimes the formation of a precipitate can be prevented by the use of a protective colloid.

Conditions for a Colorimetric Method

The following is a list of some of the desirable features which can be cited for an ideal colorimetric method. About the only one that is followed in practice is that a color is developed.

1. The color developed from a small amount of test substance should be intense.

2. The color developed should be stable.

3. The color preferably should be little affected by pH. If the color is affected by pH a buffer may be used to control the pH.

4. For visual colorimetric methods the transmission should be between 475-625 millimicrons. The range of photoelectric methods depends upon the range of sensitivity of the photocell.

5. Temperature should have little effect on the color.

6. When a system follows Beer's law, the standards and calibration curves may be obtained more easily.

7. It is desirable to have the color develop quickly.

8. The reaction between the test substance and reagents should be specific. The more specific the reaction the less will be the number of interfering substances.

9. The order of mixing should not be critical.

10. The colored solution should require no special treatment such as extraction with a solvent.

11. The color developed is preferably independent of excess reagent.

Filter Photometry

The filter photometer is an instrument used to compare light-absorbing properties of a colored system. Filter photometers are of two types depending on the device used to indicate the comparisons: (1) visual filter photometers where the eye is used and (2) photoelectric filter photometers where a photoelectric cell is used. Modern filter photometers are generally spectrophotometers in which the expensive monochromator has been replaced by a filter. Hence the photoelectric colorimeter is a type of filter photometer.

When a photoelectric colorimeter is used under the proper conditions it is generally more sensitive than the corresponding visual methods. Also, a photoelectric colorimeter with the proper combination

of light source, photocell, and filter is sensitive in the ultraviolet, infrared, both invisible to the eye, as well as in the range 400-700 mμ where the eye can be used. When a large number of determinations of the same kind have to be undertaken, a photoelectric filter photometer will give decreased time per determination, and also be less fatiguing on the analyst. If close screening is not necessary to separate an interfering wave band, a filter photometer is as satisfactory as a true spectrophotometer and is much less expensive.

The use of a photoelectric colorimeter requires some knowledge of the color characteristics of the solution and the applicability of the laws of light absorption. The essential theory governing colorimetric analysis consists of the physical laws of light. These are Lambert's (Bouguer's) law and Beer's law.

Lambert's Law gives the relationship between color intensity and depth. This law states that at constant concentration the color intensity is directly proportional to depth.

Beer's Law involves the relationship between color intensity and concentration. The law states that at constant depth the color intensity is directly proportional to the concentration.

Derivation of Beer's Law and Lambert's Law

(a) Lambert's Law

When light passes through a unit layer of a solution, the energy of a given wavelength is reduced by a fraction of its intensity. In the next layer an identical fraction of the remaining intensity is absorbed. The decrease of intensity per increment of depth of solution is hence proportional to the intensity of the energy passing through that layer. This observation was made by Lambert in 1760. This law may be expressed by the following differential equation:

$$- \frac{dI}{dl} = kI \qquad (1)$$

where I and l represent intensity and thickness respectively. Upon rearranging the terms and integrating we get:

$$\ln I = -kl + \text{constant} \quad (2)$$

when $l = 0$, the intensity is equal to the initial intensity $I_o$, whence

$$\ln I_o = \text{constant}$$

Substituting this value in equation (2), we get:

$$\ln I_t - \ln I_o = - kl \quad (3)$$

where $I_t$ = the intensity of the transmitted light

$I_o$ = the intensity of the incident light.

By rearranging the left-hand side of equation (3) we get the logarithmic form of Lambert's law: $\ln \frac{I_t}{I_o} = -kl \quad (4)$

Lambert's law may also be expressed in the exponential form:

$$\frac{I_t}{I_o} = e^{-kl} \quad (5)$$

where $I_t$ = the intensity of the transmitted light

$I_o$ = the intensity of the incident light

$k$ = is a constant called the absorption coefficient.

$e$ = naperian base of logarithms.

If one changes from logarithms to base e to those of base 10 we get the following expression from equation (4):

$$\log \frac{I_t}{I_o} = - Kl \quad (6)$$

where $K = 0.4343\ k$

The logarithm of the reciprocal of $\frac{I_t}{I_o}$ (the transmission factor) is often referred to as the optical density, d, namely

$$d = \log \frac{I_o}{I_t} = Kl \quad (7)$$

Beer's Law: If one has a number of solutions, $S_1, S_2$-----, made up of the same solvent and solute but having the solute present in different amounts, $C_1, C_2$ ------, then it follows from Lambert's law, that for each solution one will have a transmittance T corresponding to the absorption coefficients $k_1, k_2$-----, as follows:

$$T \text{ for } C_1 = e - k_1\ l$$

$$T \text{ for } C_2 = e - k_2\ l$$

changing from base e to base 10 we get, $\cancel{10 = K_1 l}$

$$T \text{ for } C_1 = 10^{-K_1 l}$$

$$T \text{ for } C_2 = 10^{-K_2 l}$$

Beer showed that the absorption coefficients $K_1, K_2$, -----, are related to the concentiations $C_1$, $C_2$,-----. For some solutions this relationship is linear and thus we may write,

$$K = ac \quad (1)$$

where a is a constant. When this is so Beer's law is said to hold. k is sometimes called the extinction coefficient.

Solutions conforming to Beer's law have a constant molecular extinction coefficient at all dilutions and thicknesses for a given wavelength. Spectrophotometric curves (optical density vs. wavelength) for a solution obeying Beer's law should have the same shape for different concentrations and in addition the extinction coefficients should be proportional to the concentration. A curve from a spectrophotometer will have a maximum at the wavelength of maximum absorption of the system. At this point there is the largest change in transmission for a given change in concentration.

## PART 2.

Determination of Small Amounts of Fluorides

Part 2:- Determination of Small Amounts of Fluorides

Discussion of the Colored System to be Used

The bleaching effect of fluoride ion on the red lake formed by sodium alizarin sulfonate and a zirconyl salt is due to the formation of a stable complex ion, between zirconium ion and the fluoride ion. The sodium alizarin sulfonate released on the breaking down of the red lake by fluoride ion is yellow in acid solution. The red zirconium-sodium alizarin sulfonate lake is stable in acid solution. With increasing amounts of fluoride ion the color of the solution changes gradually from red to yellow.

The zirconium-fluoride complexes are colorless. Venables (31) reports that the following complexes occur:

$Zr_3F_{17}^{-5}$, $Zr_3F_{13}^{-5}$, $ZrF_5^{-}$, $ZrF_6^{--}$, $ZrF_7^{---}$.

In very dilute solutions complexes containing much less fluorine may occur.

In order to determine the optimum wavelength to use, a series of spectrophotometer curves were obtained on the red zirconium lake and the yellow sodium alizarin sulfonate. The filters are used in the photoelectric colorimeter to block off all light except a narrow band in the region of maximum absorptance. This gives a maximum change in transmittancy for a given change in concentration when the colorimeter is in use.

The visual colorimetric methods for the determination of fluorides in small quantities were investigated. Steiger (24) developed a colorimetric method based on the fading of the orange color of titanium peroxide in the presence of fluorides. This bleaching is believed to be due to the formation of a complex. Wilcox, Fahey, and Foster have devised similar methods involving the fading of the color of a ferric salt due to the formation of the $FeF_6^{---}$ complex.

Talvitie (28) has devised a method which depends upon the fading of a thorium-sodium alizarin sulfonate lake. The thorium forms an insoluble fluoride. Sanchis (21), Elvove (10) and Scott (22) have developed methods suitable for water analysis from the earlier methods of De Boer, Casares and Thompson and Taylor. These colorimetric methods depend upon the fading of the zirconium lake of a dye, sodium alizarin sulfonate, in acid soulution,by the formation of complexes. These methods differ in that they use successively a less number of reagents. Sanchis used three; Elvove used two; and Scott used one. It was decided to investigate the methods involving the use of a zirconyl-sodium alizarin sulfonate lake because of their rapidity and their lesser sensitivity to common interfering ions. The method used by Scott appears to be the most rapid.

Preliminary Experimantal Work.

(a) Sanchis Modification

It was decided upon to investigate the Sanchis modification first. This colorimetric method depends upon the fading of a zirconyl-sodium alizarin sulfonate lake by fluoride ion. In the original paper (21) comparison was made visually by use of a series of standards in Nessler tubes. This method has been adapted to use in a photoelectric colorimeter by Walker and Gainer (32) and by Murphy (18). The following is an outline of the reagents used in this method:

Preparation of the Reagents

1. Standard NaF solution: 2.21 grams of dried sodium fluoride is first dissolved in one litre of distilled water to give a stock solution containing 1000 p.p.m. of fluoride ion. 100 ml. of this solution is diluted to one litre to give a solution of 100 p.p.m. $F^-$ 100 ml. of this solution containing 100 p.p.m. of fluoride ion is diluted to one litre to give a working solution containing 100 p.p.m. of fluoride

ion.

2. The Indicator

(a) 0.17 grams of sodium alizarin sulfonate was dissolved in 100 ml. of distilled water.

(b) 0.87 grams of zirconyl nitrate $ZrO(NO_3)_2 \cdot 2H_2O$ was dissolved in 100 ml. of distilled water. Both solutions (a) and (b) are stored in the dark.

(c) In order to prepare a working solution, 10 millilitres of (a) is added to 10 ml. of (b); diluted to 100 ml. and allowed to stand for 12 hours before using.

3. 3N HCl: 250 ml. of concentrated HCl are mixed with 500 ml. of distilled water in a one litre volumetric flask. The solution is cooled to room temperature and filled up to the mark with distilled water.

4. 3N $H_2SO_4$: 84 ml. of concentrated $H_2SO_4$ are mixed with 500 ml. of distilled water in a one litre volumetric flask. The solution is cooled and diluted to one litre.

Preparation of Standards

To a series of 100 ml. volumetric flasks are added 0.0, 2.0, 4.0, 6.0, 10.0, 12.0 and 14.0 ml. of standard sodium fluoride solution. The standard NaF solution is measured out with Mohr pipettes. The 10 p.p.m. solution of NaF is used for this purpose.

Procedure

The practicability of the original Sanchis method for the determination of fluorides was first investigated. The original method is as follows:

2.0 ml. of 3N Hcl and 2.0 ml. of 3N $H_2So_4$ are added to 100 ml. of the standard along with 1.8 ml. of the indicator. The acids were measured out by means of a Mohr pipette while the indicator was measured

out with a micro-burette. The solution is heated to boiling and allowed to stand overnight. These solutions are then compared visually in Nessler tubes.

In order to determine the absorption maximum of the red lake formed in this modification, a sample of the indicator was examined in a Nutting-Hilger spectrophatometer. This is a repetition of the work done by Walker and Murphy.

Determination of the Absorption Spectrum

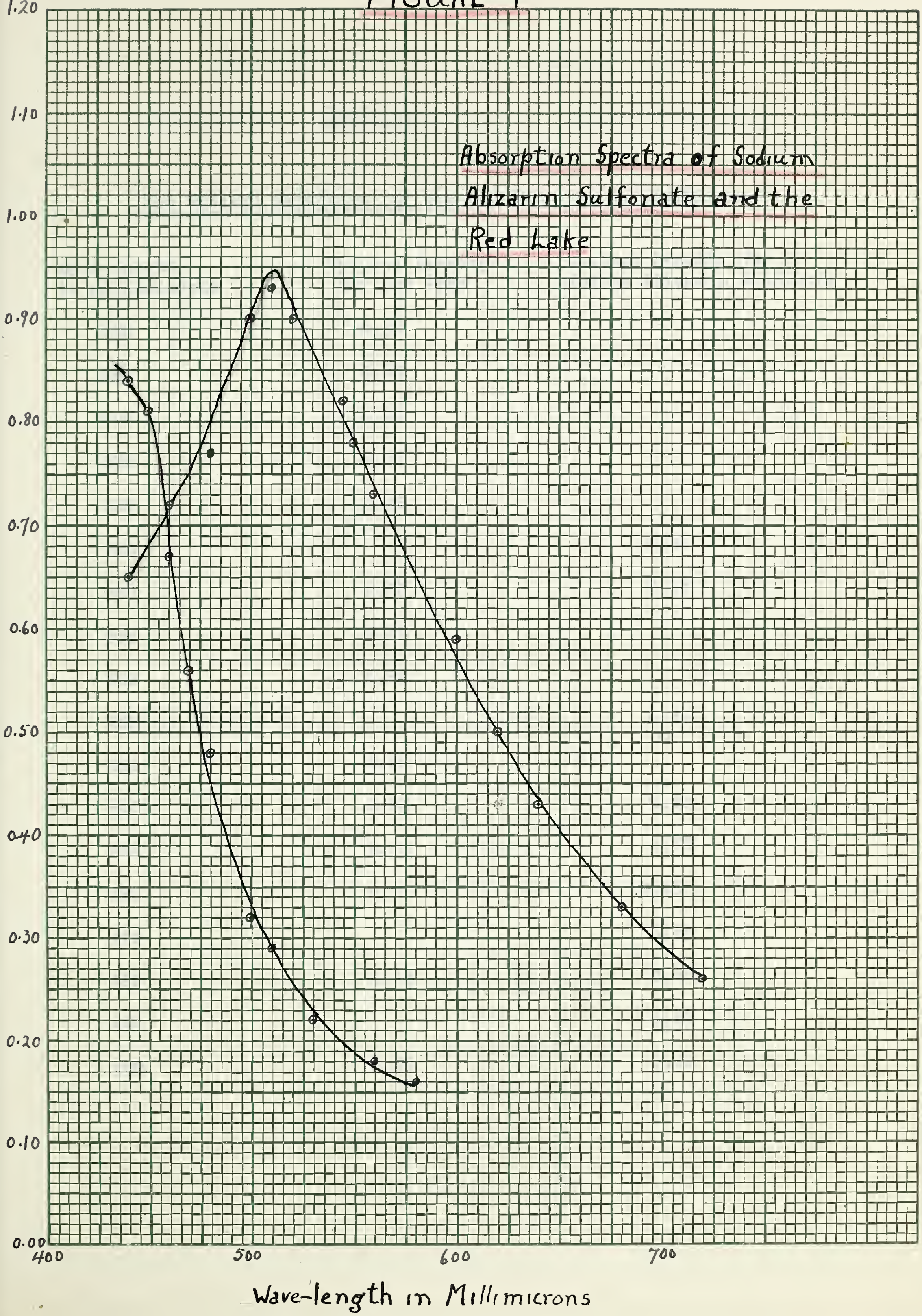
A sample of the indicator (the red lake) prepared according to the Sanchis modification was used:

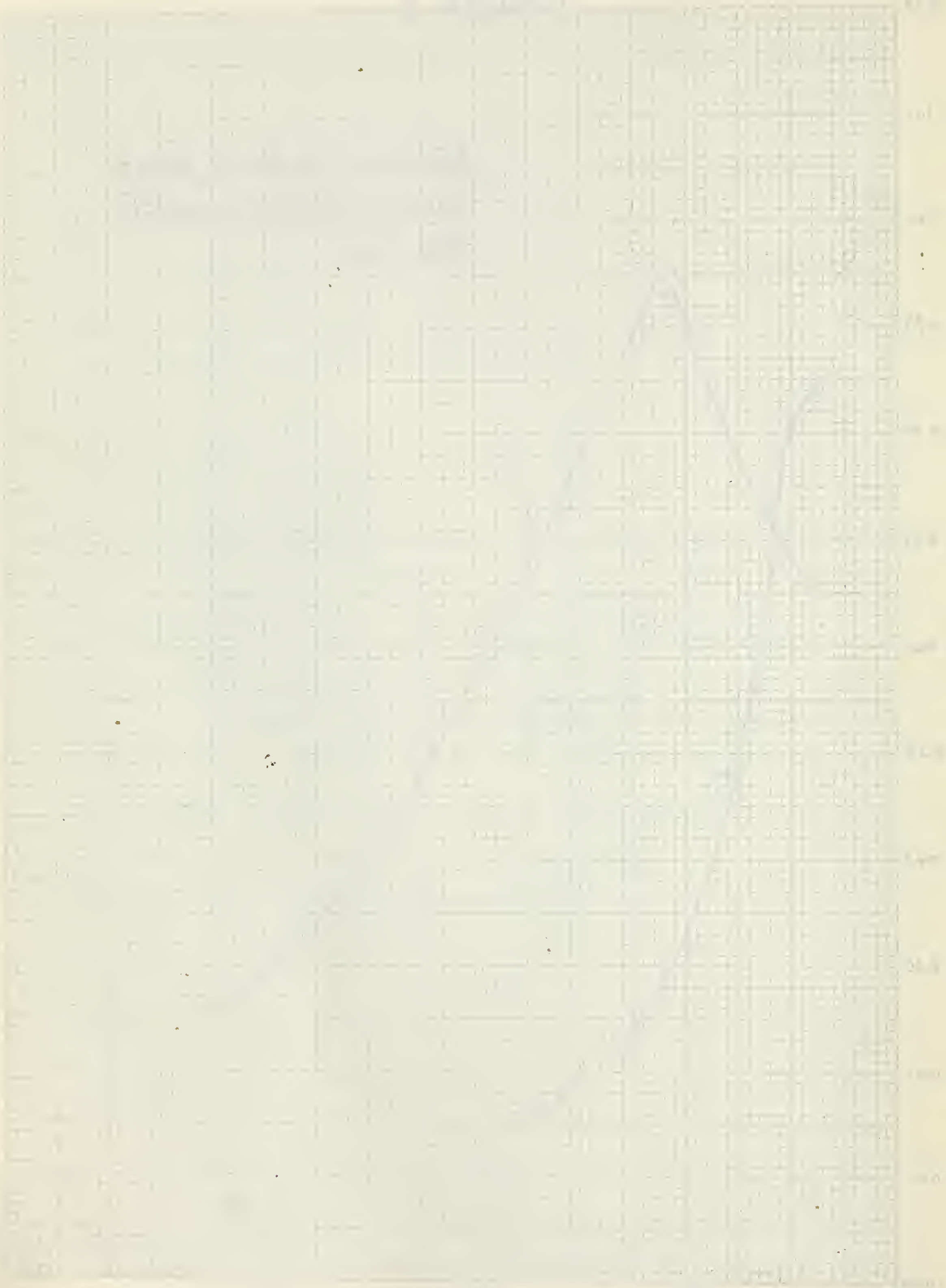
Ten ml. of this solution was mixed with 4 ml. of a mixed acid solution containing 3N $H_2SO_4$ and 3N HCL in equal amounts and the solution diluted to 100 ml. with distilled water. This solution was brought to a boil, removed after five or ten seconds boiling and allowed to stand for 24 hours before examining it in the spectrophotometer. Table (1) shows the optical densities obtained at various wavelengths. Figure (1) contains the curve obtained by plotting optical density against wavelength in millimicrons (Curve A). It can be seen that the maximum absorption occurs between 515 and 520 millimicrons.

The fluorine bleaches the red lake by forming a stable complex ion with zirconium and releasing the sodium alizarin sulfonate which is yellow in acid solution. Since interference in the photoelectric colorimeter with the red lake might be caused by this yellow colored compound the absorption spectrum of sodium alizarin sulfonate of the same concentration as used in the indicator was determined in the same spectrophotometer. The result is given as curve B in figure (1). Although the two curves overlap, the sodium alizarin sulfonate has its absorption maximum in the ultraviolet region of the spectrum.

In using the photoelectric colorimeter, the light filter to be selected is one that screens out all wavelengths except those in a narrow

FIGURE 1


Absorption Spectra of Sodium Alizarin Sulfonate and the Red Lake
1.20
1.10
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
400
500
600
700
Wave-length in Millimicrons

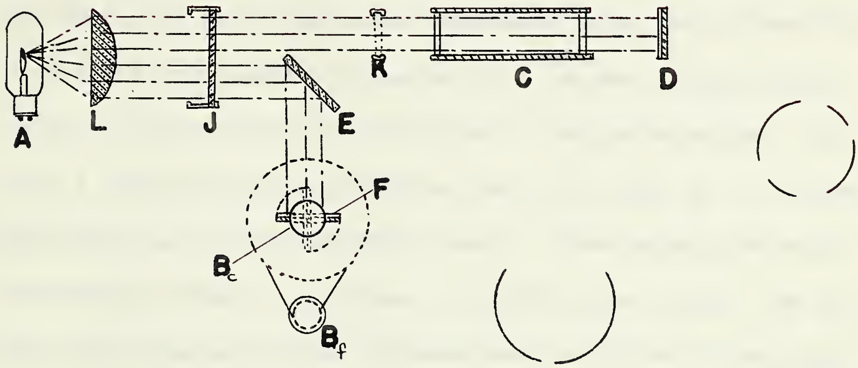


## Table I

The Optical Densities for the Red Lake and Yellow Dye

| Wave length in millimicrons | Optical Density for Red Lake | Optical Density for Sodium Alizarin Sulfonate |
|---|---|---|
| 720 | 0.26 | |
| 680 | 0.33 | |
| 640 | 0.43 | |
| 620 | 0.50 | |
| 600 | 0.59 | |
| 580 | | 0.16 |
| 560 | 0.73 | 0.18 |
| 550 | 0.78 | |
| 545 | 0.82 | |
| 530 | | 0.22 |
| 520 | 0.90 | |
| 510 | 0.93 | 0.29 |
| 500 | 0.90 | 0.32 |
| 480 | 0.77 | 0.48 |
| 470 | | 0.56 |
| 460 | 0.72 | 0.67 |
| 450 | | 0.81 |
| 440 | 0.65 | 0.84 |

# THE OPTICAL SYSTEM OF THE
## *LUMETRON PHOTOELECTRIC COLORIMETER*
## — MODEL 402-E —




| PART NUMBER | NAME |
|---|---|
| A | LIGHT SOURCE. |
| $B_c$ | COARSE ADJUSTMENT. |
| $B_f$ | FINE ADJUSTMENT. |
| C | ABSORPTION CELL. |
| D | MEASURING PHOTOCELL |
| E | MIRROR. |
| F | BALANCE PHOTOCELL. |
| J | FILTER HOLDER. |
| K | CLAMP. |
| L | COLLIMATING LENS. |

FIGURE 2.

band near the absorption maximum; the effect of the sodium alizarin sulfonate will be small and will not cause much interference. It is not likely, though, that the system will follow Beer's law.

The Lumetron Photoelectric Colorimeter Model 402-E.

The instrument was manufactured by the Photovolt Corporation, New York. It is a null point instrument using two photocells, a slide wire and a galvanometer connected in a current bridge circuit. The balance of the circuit is indicated by the galvanometer. The light from a 100 candlepower projection lamp, A, (fig. 2) is collimated by the lens, L, to form a parallel beam. After passing through a monochromatic filter, J, the beam is split in two parts. One part of this beam passes throught the developed solution in the sample holder, C, and strikes the measuring photocell, D. The other part of the beam is deflected by a mirror, E, to act on the balance photocell, F, which is mounted so that it may be rotated through an angle of $90^{\circ}$. When the active face of the balance photocell is normal to the beam the cell receives the full light. When the balance cell is rotated to the other stop position the edge of the active face is presented to the light and there is no action on the cell. Therefore the light striking the balance photocell may be varied by turning the knobs $B_c$ (coarse) and $B_f$ (fine) to set the galvanometer to zero.

The galvanometer is a taut-wire, spot light instrument with a coil resistance of 90 ohms and a sensitivity of 0.06 microamperes per division. It is sturdy and requires no special care as far as vibrations are concerned. The spot-light readings are free from parallax and permit adjustment to true zero. The balancing of the bridge is achieved by turning the adjustable contact arm of a slide-wire which is provided with a calibrated dial. The slide wire has a coarse and fine adjustment. The slide-wire consists of a large number of turns

of low resistance wire. The slide-wire is 9 inches long and is calibrated in percentages. It can be read to a fraction of one percent provided the sensitivity of the instrument produces a clearly detectable off-balance on the galvanometer for such small changed of the slide wire scale.

The photocells are of the self-generating barrier-layer type. These cells convert light energy directly into electrical energy. The life of these cells is unlimited and their stability has been proved by years of use. The spectral sensitivity for the cells has a maximum at very nearly the same place as that for the human eye. The cells respond to light in the range 200 to 800 millimicrons. This allows the instrument to be used in the ultraviolet when provided with a suitable light source. The light falling on the photocells, in normal operation, is usually less than one foot-candle. At such low values the response of the photocells is linear. The linearity offers the advantage that straight line plots are obtained in calibrating the instrument. For example, when measuring chemical concentration by transmission measurements, solutions obeying Beer's law give a straight line when plotted on semi - log paper.

The light source is a 100 candle-power projection lamp. The light intensity of the lamp is adjustable by a rheostat. A switch on the instrument throws the balancing cell out of the circuit. This allows the instrument to be used as a direct reading instrument. The lamp rheostat is adjusted so that a predetermined galvanometer reading is obtained in this position of the switch. This method of adjusting the light intensity offers the advantage of compensating automatically for the deterioration of the lamp. The lamp adjustment also compensates for the difference in light transmission of the various filters being used.

Each of the monochromatic filters supplied with the Lumetron Photoelectric Colorimeter consists of 2 or 3 ground and polished colored glasses. These filters isolate wave-bands approximately 30 millimicrons wide.

The absorption cells, supplied in different sizes with the apparatus have plane, parallel windows and are fused together so as to be resistant to any liquid which does not attack glass. The cells are available in rectangular shape or in cylindrical shape with filler neck. The rectangular cells are easier to clean. The cylindrical cells require less care in handling to prevent spilling.

Calibration of the Photoelectric Colorimeter for the Determination of Fluoride

The colorimeter described above was used with a 515 millimicron band filter chosen because of the curves presented in Fig. I. The sample holder was a cylindrical cell with a volume of 120 ml. and a light path of 150 mm.

The cell, of light path 150 mm., was chosen because of the experience gained by Walker and Gainer (32) in adapting a direct reading type of photoelectric colorimeter, of the type designed by Evelyn, to the determination of fluorides by the Sanchis method. They found, that the usual 7/8 inch light path used with the above colorimeter, was not sensitive enough for the determination of fluorides by the Sanchis modification. They used a Nessler tube with a light path of 21.5 centimetres and then got results similar to those obtained by visual methods. A cell, of light path 150 mm., was used by Murphy (18) who used a Lumetron Photoelectric Colorimeter in the determination of fluorides by this method.

A set of standards were made up as for the Sanchis method already given. These contained 0.0, 2.0, 4.0, 6.0, 8.0, 10.0, 12.0 and 14.0 ml. of standard NaF solution corresponding to 0.0, 0.2, 0.4, 0.6,

FIGURE 3

Calibration Curve for
Sanchis Method Modified
82.0
80.0
70.0
60.0
50.0
40.0
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
p.p.m. F⁻

0.8, 1.0, 1.2 and 1.4 p.p.m. of $F^-$. These solutions were heated to boiling and allowed to stand overnight.

The transmission standard used in obtaining the readings in Table (II) was distilled water. A volume of 106 ml. of distilled water was placed in the absorption cell and the instrument was adjusted to read 100% transmission for this transmission standard. The standards, containing the prescribed amount of acid and indicator, were then compared in the photoelectric colorimeter.

A curve, Fig. (3) was constructed by plotting percentage transmission vs. p.p.m. fluoride ion. The data from which the curve was drawn are given in Table (II). The range of scale readings is 37.6. The gradation of color of the developed standards is satisfactory.

Table II

| p.p.m. $F^-$ | Percentage Transmission |
|---|---|
| 0.0 | 43.0 |
| 0.2 | 48.0 |
| 0.4 | 51.8 |
| 0.6 | 56.4 |
| 0.8 | 62.2 |
| 1.0 | 68.8 |
| 1.2 | 74.4 |
| 1.4 | 80.6 |

It was decided to drop any further investigation of the Sanchis modification due to the fact that it required considerable time. It is believed that the Scott modification, which is much more rapid and simple in operation, should behave similarly to interfering ion as the Sanchis modification. Therefore, all further investigation has been carried out using the Scott modification.

## The Scott Modification for Determining Fluorides

The practicability of the Scott modification for the determination of fluorides was investigated in so far as its applicability to photoelectric colorimetry. The method of Scott offers these three advantages over the earlier Sanchis method:

(a) There is only one reagent in place of three.

(b) The decolorization of the lake occurs rapidly at room temperature.

(c) The final colors are persistently clear and no precipitation of the lake occurs.

## Preparation of Reagents

(a) Standard NaF Solution: same as for the Sanchis modification.

(b) 0.07 gm. of sodium alizarin sulfonate is dissolved in 50 ml. of distilled water.

(c) 0.30 gm. of zirconium oxychloride is dissolved in 50 ml. of distilled water giving a clear solution.

(d) Mixed Acid Solution:

1. 2.7 N $H_2SO_4$: 75 ml. of concentrated $H_2SO_4$ are pipetted into a one litre volumetric flask containing approximately 500 ml. of distilled water. The solution is cooled and then brought up to the mark with water.

2. 2.7 N HCl: 225 ml. of concentrated HCl is pipetted into a one litre volumetric flask containing approximately 500 ml. of distilled water. This solution is allowed to cool and then diluted to the mark with water.

   The 2.7 N HCl and 2.7 N $H_2SO_4$ are mixed to give the "mixed acid" solution.

(e) The Indicator: The Zirconium oxychloride solution (b) is transferred to a one litre volumetric flask and the sodium alizarin sulfonate solution (A) is added slowly with constant mixing. The red lake is allowed to form for fifteen minutes and then the volumetric flask is filled to the mark with mixed acid solution. The indicator is red at first but changes to a yellow color when it is ready for use (about one hour).

## Procedure

The transmission standard used, was distilled water. A volume of 105 ml. of distilled water. A volume of 105 ml. of distilled water was placed in the 15 cm. absorption cell and the instrument was adjusted to read 100% transmission ~~transmittance~~ for this transmission standard.

A series of standards was prepared containing from 0.0 to 1.4 p.p.m. of fluoride.

The standards, containing the prescribed amount of indicator, were compared in the photoelectric colorimeter. The effect of using varying amounts of the Scott indicator in the calibration of a series of standards was investigated. Sets of standards were compared in the colorimeter using 2,3,4 and 5 ml. of indicator. The results are shown in Table III. The same lot of indicator was used for all the comparisons. The calibration curves are shown in figure 4 where scale readings are plotted vs. p.p.m.F-.

TABLE III

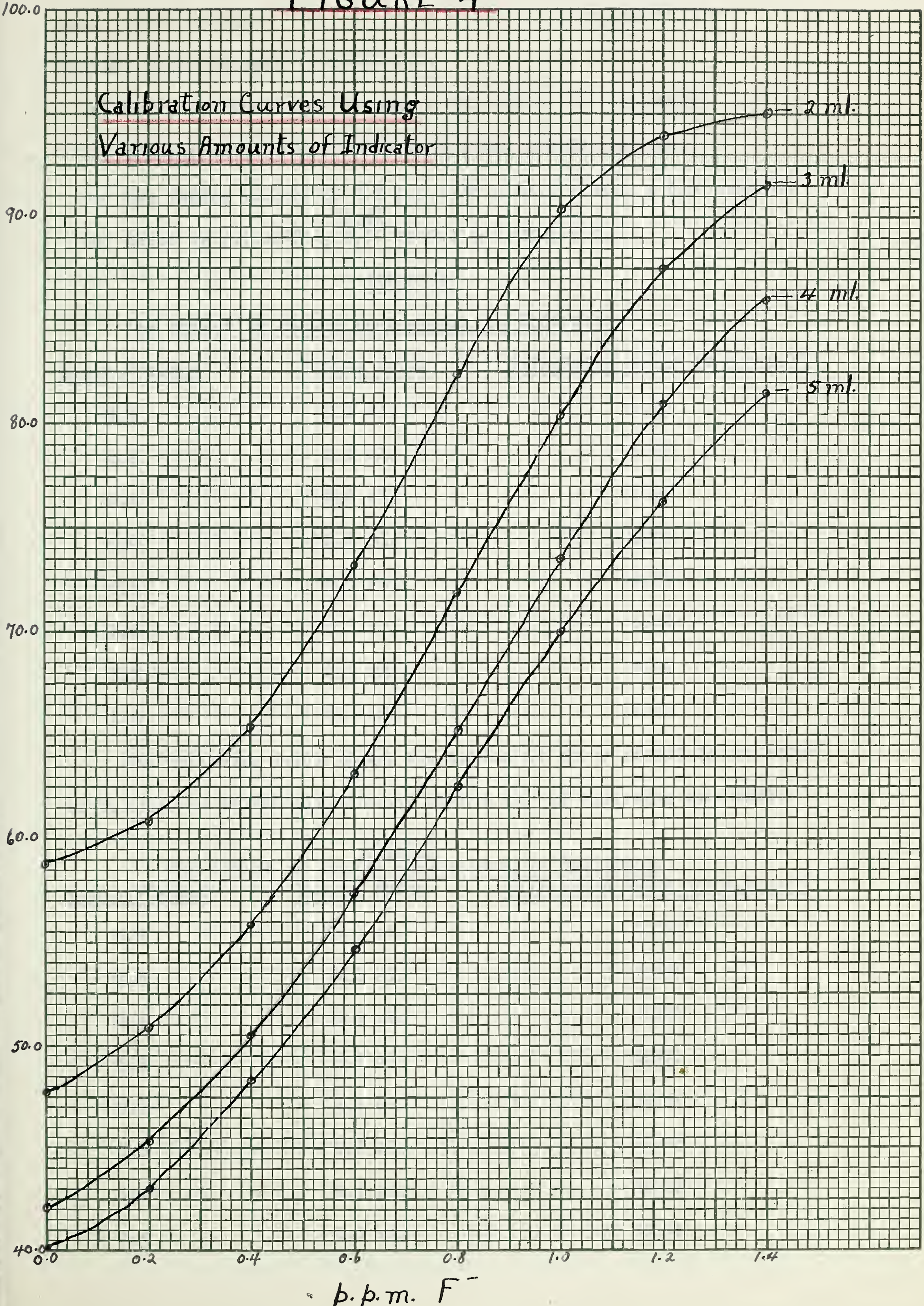
Calibration of the Colorimeter Using Varying Amounts of Indicator

Percentage Transmission

| p.p.m.F- | 2 ml. of indicator | 3 ml. of indicator | 4 ml. of indicator | 5 ml. of indicato |
|---|---|---|---|---|
| 0.0 | 58.8 | 47.7 | 42.1 | 40.1 |
| 0.2 | 60.8 | 50.8 | 45.3 | 43.0 |
| 0.4 | 65.4 | 55.8 | 50.5 | 48.4 |
| 0.6 | 73.2 | 63.2 | 57.4 | 54.7 |
| 0.8 | 82.4 | 71.9 | 65.2 | 62.5 |
| 1.0 | 90.3 | 80.4 | 73.5 | 70.0 |
| 1.2 | 93.9 | 87.5 | 81.0 | 76.3 |
| 1.4 | 95.0 | 91.5 | 86.0 | 81.5 |
| Range of Readings: | 36.2 | 43.8 | 43.9 | 42.1 |

The varying amount of indicator has the effect of shifting the range within which the percentage transmissions occur. For example, the readings obtained, when two ml. of indicator are used, lie between 58.8 and 95.0; while, when 5 ml. of indicator are used they lie between 39.4 and 81.5. From this shift an estimate of the error introduced by faulty measurement of the volume of indicator added to the stand-

FIGURE 4



Calibration Curves Using
Various Amounts of Indicator
2 ml.
3 ml.
4 ml.
5 ml.
100.0
90.0
80.0
70.0
60.0
50.0
40.0
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
p.p.m. F⁻

ards can be obtained. The error in table IV is calculated as follows from the data in table III:

(a) the shift caused by one ml. = 42.1-39.4 = 2.7 percent.

(b) the approximate shift due to 0.1 ml. = 0.27 percent.

(c) the fading caused by 0.2 p.p.m.$F^-$ = 43.0 - 40.0 = 3.0 percent.

(d) error in p.p.m.$F^-$ = $\frac{0.27}{3.0}$ of 0.2 = 0.02 p.p.m.

TABLE IV

The Effect of Faulty measurement in Indicator.

| p.p.m.$F^-$added | Volume Error | Calculated Error in p.p.m.$F^-$ |
|---|---|---|
| 0.0 | 0.1 ml. | 0.02 |
| 0.2 | 0.1 ml. | 0.01 |
| 0.4 | 0.1 ml. | 0.01 |
| 0.6 | 0.1 ml. | 0.01 |
| 0.8 | 0.1 ml. | 0.01 |
| 1.0 | 0.1 ml. | 0.01 |
| 1.2 | 0.1 ml. | 0.02 |
| 1.4 | 0.1 ml. | 0.02 |

The following table shows the estimated error caused by an error of 0.1 ml. in volume measurement when 4 ml. of indicator are used.

TABLE V

The Effect of Faulty measurement in Indicator.

| p.p.m.$F^-$added | Volume Error | Calculated Error in p.p.m.$F^-$ |
|---|---|---|
| 0.0 | 0.1 ml. | 0.04 |
| 0.2 | 0.1 ml. | 0.02 |
| 0.4 | 0.1 ml. | 0.02 |
| 0.6 | 0.1 ml. | 0.01 |
| 0.8 | 0.1 ml. | 0.02 |
| 1.0 | 0.1 ml. | 0.02 |
| 1.2 | 0.1 ml. | 0.02 |
| 1.4 | 0.1 ml. | 0.02 |

When five ml. of indicator are used the error introduced by small deviations in volume measurement is small. This error is greater when only 4 ml. of indicator are used. Due to this small error the accuracy of a Mohr pipette is sufficiently good for these determinations.

From table III one may observe that the range in percentage transmissions (gradation in color) is a little better when either 3 or 4 ml. of indicator are used. This slight change in range is probably a combination of the effect caused by a change in pH and the effect brought about by using less of the colored constituent. The interference of sulfate ion is probably greater when less amounts of the indicator are used. Therefore, in all future determinations and calibrations 5 ml. of the indicator have been used.

Several different lots of the indicator were prepared over a period of time using identically the same amount of reagents, order of mixing and time of mixing. The photoelectric colorimeter was calibrated with great care for each of these lots of indicator. No two of these calibrations were exactly the same. Therefore, it is considered necessary to calibrate the photoelectric colorimeter each time a new lot of indicator is made up. The variation in the readings for each separate lot of indicator is tabulated in table VI. The calibration curves are shown in figure 5.

It has been observed that it is better to allow a freshly prepared lot of the indicator stand from one to two days before use. For the first two days there appears to be a slight change taking place in the indicator. A calibration curve mow may be used for several weeks if the same lot of indicator isused. Even so, frequent checks of the calibration should be made by determing one of the standards in the photoelectric colorimeter. If the change is very great, the colorimeter should be re-calibrated.

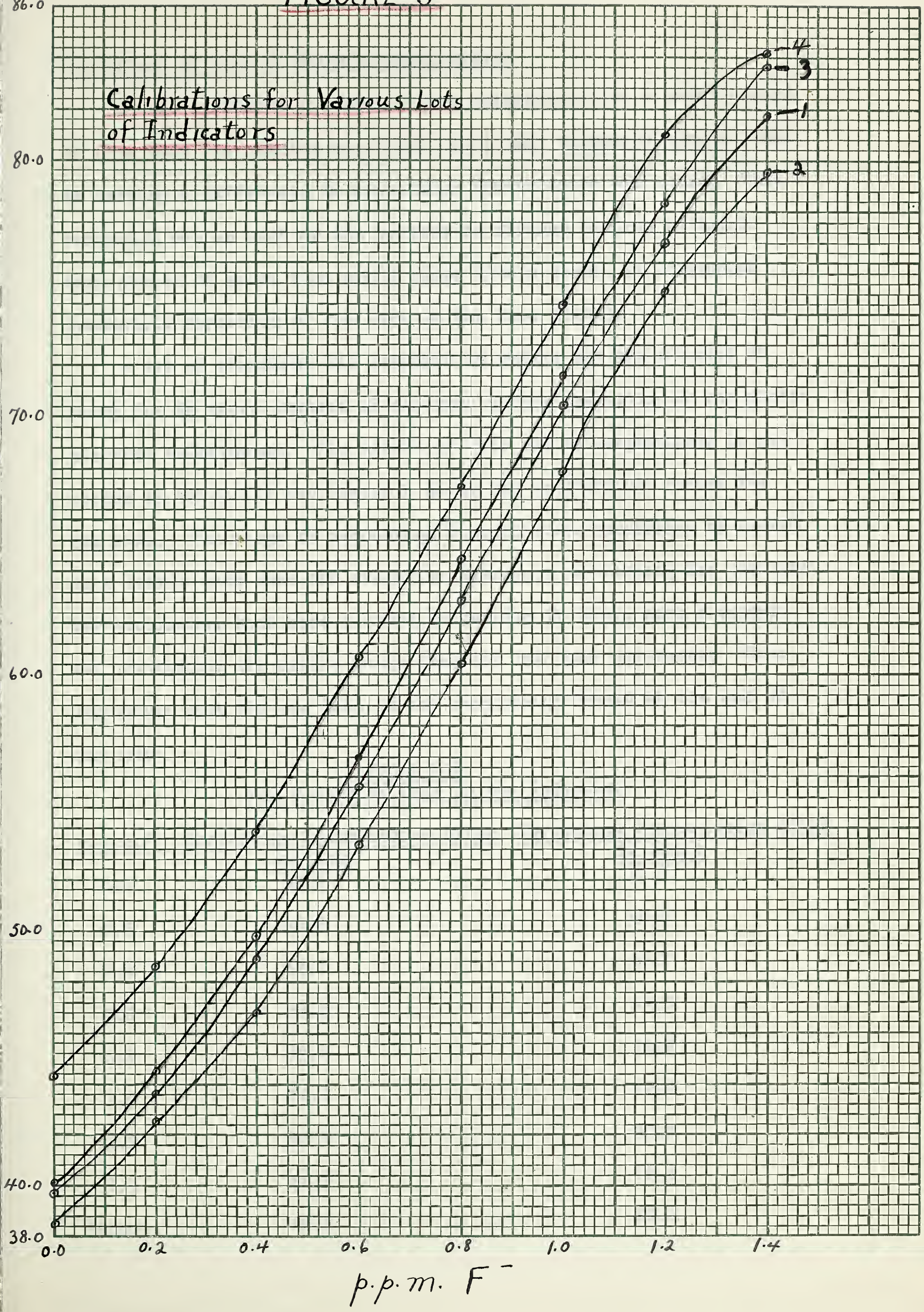
TABLE VI

CALIBRATION AND INDICATOR LOT

| p.p.m$F^-$ | Indicator Lot No. | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| 0.0 | 39.7 | 38.5 | 40.1 | 44.3 |
| 0.2 | 43.6 | 42.5 | 44.5 | 48.6 |
| 0.4 | 48.9 | 46.8 | 49.8 | 53.9 |
| 0.6 | 55.6 | 53.4 | 56.8 | 60.7 |
| 0.8 | 62.9 | 60.4 | 64.5 | 67.3 |
| 1.0 | 70.5 | 67.9 | 71.6 | 74.4 |
| 1.2 | 76.8 | 74.9 | 78.3 | 81.0 |
| 1.4 | 81.8 | 79.5 | 83.6 | 84.2 |

-.-.-.-.-.-.-.-.-.-.-.-.-.-.-.-.-.-

FIGURE 5


Calibrations for Various Lots
of Indicators
86.0
80.0
70.0
60.0
50.0
40.0
38.0
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
4
3
1
2
p.p.m. F⁻

# FIGURE 6

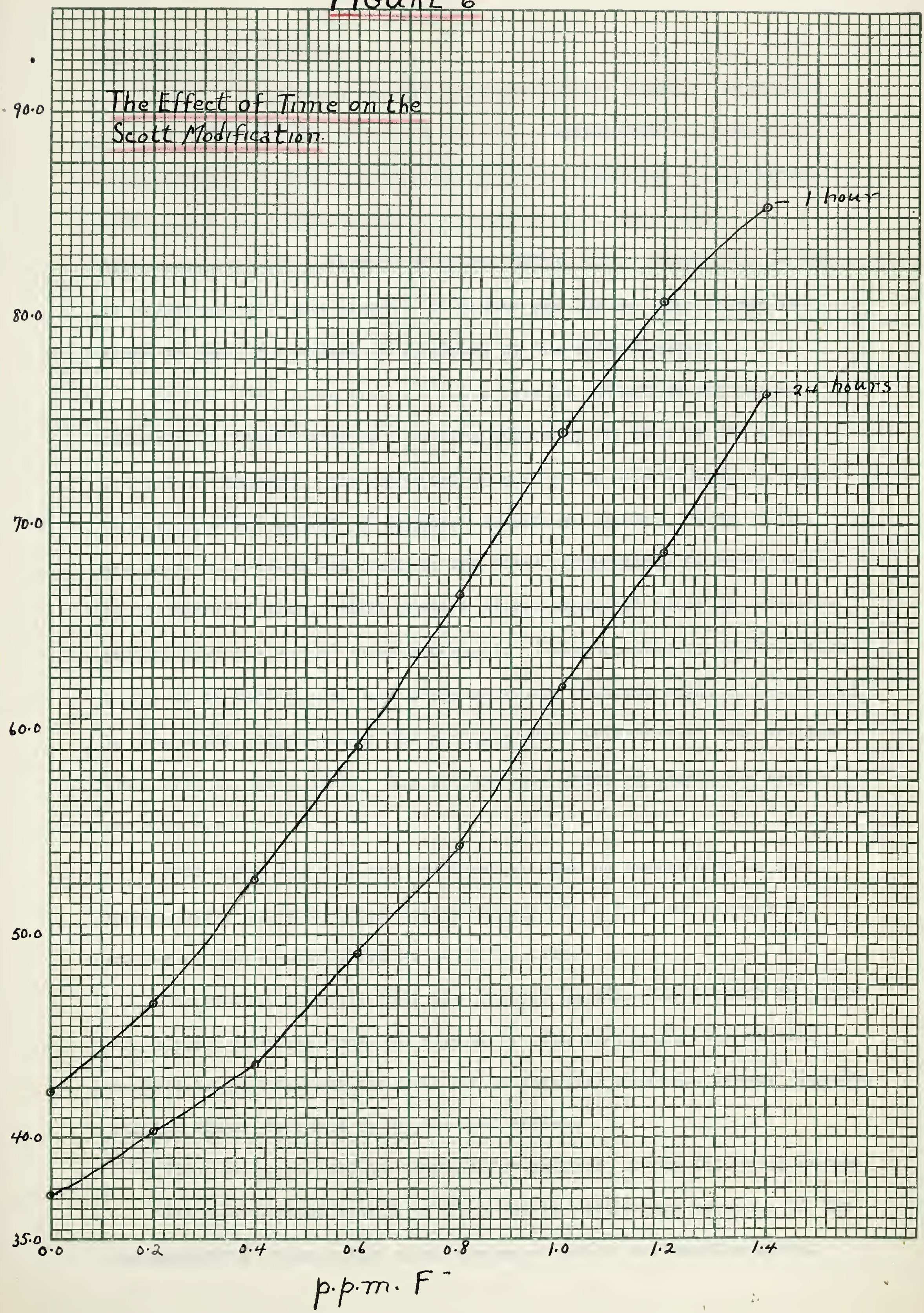
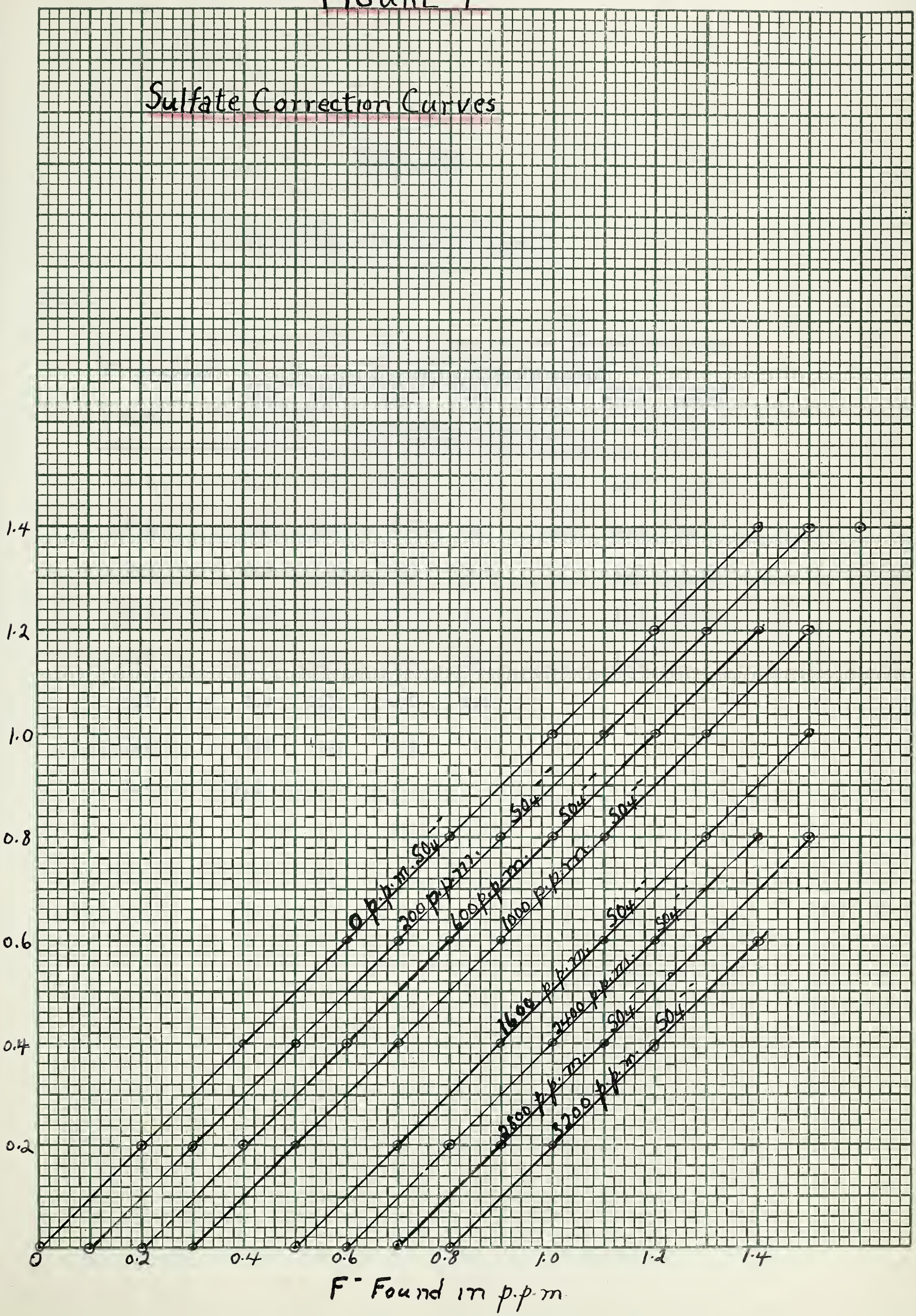

The Effect of Time on the Scott Modification
1 hour
24 hours
90.0
80.0
70.0
60.0
50.0
40.0
35.0
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
p.p.m. F⁻

FIGURE 7



Sulfate Correction Curves
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
0 p.p.m. SO4--
200 p.p.m. SO4--
600 p.p.m. SO4--
1000 p.p.m. SO4--
1600 p.p.m. SO4--
2400 p.p.m. SO4--
2800 p.p.m. SO4--
3200 p.p.m. SO4--
F- Found in p.p.m.

TABLE VIII

The Sulfate Correction Chart.

| $F^-$ present p.p.m. | p.p.m. sulfates added 200 | 600 | 1000 | 1600 | 2400 | 2800 | 3200 |
|---|---|---|---|---|---|---|---|
| | $F^-$ found in p.p.m. | | | | | | |
| 0.0 | 0.1 | 0.2 | 0.3 | 0.5 | 0.6 | 0.7 | 0.8 |
| 0.2 | 0.3 | 0.4 | 0.5 | 0.7 | 0.8 | 0.9 | 1.0 |
| 0.4 | 0.5 | 0.6 | 0.7 | 0.9 | 1.0 | 1.1 | 1.2 |
| 0.6 | 0.7 | 0.8 | 0.9 | 1.1 | 1.2 | 1.3 | 1.4 |
| 0.8 | 0.9 | 1.0 | 1.1 | 1.3 | 1.4 | 1.5 | |
| 1.0 | 1.1 | 1.2 | 1.3 | 1.5 | | | |
| 1.2 | 1.3 | 1.4 | 1.5 | | | | |
| 1.4 | 1.5 | | | | | | |

The concentration of fluorine found is converted to the concentration of fluorine present by referring to figure 7 and reading the true fluorine content from the ordinate which crosses the nearest sulfate concentration line. Corrections are generally 0.1 p.p.m. for every 300 p.p.m. of sulfate ion present in excess of the concentration of sulfate ion added as $H_2SO_4$ for the lower amounts of fluoride and approximately 400 p.p.m. sulfate requires a 0.1 p.p.m. fluoride correction for the higher concentrations of fluorine.

The tetrahydroquinone method for sulfate determination as outlined in Betz:"Handbook of Industrial Water Conditioning", 1945,p.145, is used for the determination of sulfate ion. The sodium salt of tetrahydroquinone is yellow in the neutral ethanol-water solution and turns a rose-red in the presence of an excess of barium ion.

A standard $BaCl_2$ solution is added to the unknown sulfate solution and the endpoint of the titration is a change from yellow to red. A blank should always be run.

Interfering Ions

According to Scott's original method, any method of fluorine determination involving the decolorizing of a lake should include a check on interfering ions. He found that up to 250 p.p.m. sulfate, the higher concentration of sulfate added as sulfuric acid minimized the effect such that no correction was necessary. Every 100 p.p.m. of sulfate in excess of this caused an apparent increase of 0.05 p.p.m. fluorine. The writer of this paper has found a correction of 0.1 p.p.m. fluorine necessary at 200 p.p.m. of sulfate and 300 p.p.m. of sulfate was necessary to cause an apparent increase in fluorine content of 0.1 p.p.m. This is lower than that found by Scott.

The interference of chloride is greatly suppressed by the addition of H Cl (2).

Taylor and Frazier (29) studied the effect of phosphates on the colorimetric determination of fluorine and found the interference greater with metaphosphates than with orthophosphates. Since orthophosphates are met with more frequently in water than other phosphates we shall now use the term, phosphate, when referring to orthophosphate. The interference decreases with the increasing hardness of water, probably due to the action of calcium and magnesium with the phosphates.

The Effect of Phosphates.

It is wellknown that phosphates interfere with the determination of fluorides in all of the reported colorimetric and volumetric methods. (7). Most natural waters do not contain sufficient phosphate to interfere but foods contain phosphate in greater amounts and the separation of phosphate from the fluoride becomes necessary for accurate fluorine determinations.

The phosphates and fluorides can be separated by distilling with perchloric acid when organic material is absent (36). When organic material is present it has been found necessary to carry out two separate distillations in order to separate the phosphate and fluoride. The first distillation is carried out with concentrated sulfuric acid; this distillation is followed by a distillation with perchloric acid(8). These distillations require special apparatus and are very time consuming. A much quicker method for the determination of fluorine in the presence of phosphate is required.

It was, therefore, decided to investigate the effect of phosphate on the Scott modification and observe whether or not a correction chart similar to that obtained for sulfates could be obtained for phosphates. It is well known that soluble zirconium salts form a precipitate with phosphate ion that is insoluble in acid solution.

Phosphates are reported to interfere with the Scott modification when present in concentrations as low as 1 p.p.m.

Known amounts of phosphate were added to standards prepared as before and their apparent fluorine content determined by the method of Scott using the photoelectric colorimeter. The results are given in table IX. The phosphate was added as potassium dihydrogen phosphate, c.p., which had been dried over $P_2O_5$.

1.43 grams of $KH_2PO_4$ were dissolved in one litre of water to give a stock solution of phosphate containing 1000 p.p.m. $PO_4^{---}$. More dilute solutions were obtained in the same manner as that used for standard fluorine solutions.

From table IX it can be observed that no correction is necessary until one has added 2 p.p.m of phosphate ion. When the concentration of fluoride is small (0.0 - 0.2 p.p.m.) the interference of phosphate is more marked than when the concentration of fluoride is in the range, 0.2 - 0.8 p.p.m. The interference of phosphate again becomes more marked after the concentration of fluoride becomes greater than 0.8 p.p.m.

The Scott method is quite good up to 4 p.p.m. of phosphate and the correction chart can be used. The effect of phosphate has been found to be somewhat erratic at different times. The determination of fluoride in a sample containing very much phosphate should never be taken on only one sample.

Since phosphates interfere with fluorine determinations; a rapid, accurate method for the determination of phosphates is of prime importance in this work. A rapid photoelectric colorimetric method for the determination of phosphates will be discussed in Part 3 of this thesis.

<u>Fluorides in Edmonton City Dometic Water Supply</u>

The fluoride content of the water supply of the City of Edmonton

[illegible]

Table IX

Chart of Phosphate Interference

| $F^-$ present p.p.m | p.p.m. phosphate added 0.2 | 1.0 | 2.0 | 4.0 | 10.0 | 15.0 | 20.0 | 30.0 |
|---|---|---|---|---|---|---|---|---|
| | $F^-$ found in p.p.m. | | | | | | | |
| 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.1 | 0.1 | 0.3 | 0.3 |
| 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.3 | 0.3 | 0.3 | 0.5 |
| 0.4 | 0.4 | 0.4 | 0.4 | 0.4 | 0.5 | 0.4 | 0.5 | 0.7 |
| 0.6 | 0.6 | 0.6 | 0.6 | 0.6 | 0.6 | 0.6 | 0.7 | 1.0 |
| 0.8 | 0.8 | 0.8 | 0.8 | 0.8 | 0.8 | 0.7 | 0.8 | 1.4 |
| 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 0.8 | 0.9 | |
| 1.2 | 1.2 | 1.2 | 1.2 | 1.2 | 1.1 | 1.0 | 1.0 | |
| 1.4 | 1.4 | 1.4 | 1.4 | 1.3 | 1.2 | 1.1 | 1.0 | |

.-.-.-.-.-.-.-.-.-.-.-

has been determined periodically. Determinations have been made on both tap water and water directly from the North Saskatchewan River. Determinations have been made using the Scott modification by photoelectric means and visual comparison. The findings are given in table X. The amount of phosphate present was determined by the method outlined in Part 3 of this report.

TABLE X

Fluorides in Edmonton City Water.

| Method Used | Date | Source of Sample | p.p.m. $F^-$ | p.p.m. $SO_4^{--}$ | p.p.m. $PO_4^{---}$ | corrected $F^-$ p.p.m. |
|---|---|---|---|---|---|---|
| Photoelectric | 3/8/49 | Tapwater | 0.2 | 112 | | 0.2 |
| Visual | 16/2/50 | Tapwater | 0.1 | 172 | 0.8 | 0.1 |
| Visual | 16/2/50 | riverwater | 0.2 | 161 | 0.8 | 0.2 |
| Photoelectric | 23/3/50 | Tapwater | 0.1 | 179 | 0.7 | 0.1 |
| Photoelectric | 23/3/50 | riverwater | 0.1 | 154 | 0.8 | 0.1 |

Conclusions

1. Both the Sanchis and Scott modifications are applicable to the photoelectric colorimeter.
2. Sulfates do not interfere in the determination of fluorides by this method up to 150 p.p.m. but when sulfates are present in quantities greater than this, every 300 p.p.m. of sulfate causes an apparent increase in fluoride concentration of 0.1 p.p.m.
3. Phosphates do not interfere when present in concentrations less than and including 2 p.p.m. They interfere only slightly when present in quantities up to 20 p.p.m. The effect of phosphates is erratic at times and therefore more than one determination should be made when phosphates are present to ensure good results.
4. Scott's modification has the following advantages over the Sanchis modification:

(a) Only one reagent is required instead of three as in the Sanchis method.

(b) The Scott method is much quicker, requiring only one hour as compared to overnight for the Sanchis method.

5. It has been found necessary to re-calibrate the photoelectric colormeter each time a new lot of the Scott indicator is made up.

6. Better results are obtained by the Scott modification if the indicator is allowed to stand 48 hours after preparation before it is used. When this is done a calibration may last for two weeks but it is advisable to check the calibration from time to time by comparing a solution of known fluoride content in the photoelectric colorimeter.

## Part 3

### The Determination of Small Amount of Phosphates

Introduction

A method for the rapid determination of small amounts of phosphates is of prime importance in this research. Phosphates interfere greatly in all the existing colorimetric methods for the determination of fluorides in small quantities.

Under suitable conditions molybdates react with phosphates to form heteropoly compounds, such as ammonium molybdiphosphate, $(NH_4)3\ [P\ (Mo_3O_{10})4]$. This complex after controlled reduction to give molybdenum blue serves for the colorimetric determination of phosphorus. The procedure must be carefully controlled, as the excess molybdate reagent itself may be reduced to molybdenum blue.

The nature and composition of the material obtained on reducing simple molybdates are uncertain(15). Low concentrations of the reactants yield what appears to be a solution, but some workers indicate that the solution is colloidal (17,20).

The reduction product of heteropoly compounds is also uncertain. Two recent authors claim that the phosphorus in the molybdiphosphates acts only as a catalyst. (37).

There have been many reducing agents used to reduce the heteropoly complex. Denigès (9) used chlorostannous acid under conditions to reduce only the heteropoly acid. Bell and Doisy recommended hydroquinine as a reductant (5). Fiske and Subbarrow used aminonaphthosulfonic acid as a reductant. Feigl (13) used benzidine in ammoniacal solution as a reductant. Tartaric acid prevents the interference of arsenates.

Stoloff (27) has proposed a modification of the Association Official Agricultural Chemical method (4), which he states gives a stable blue color. He claims that both the stability and color intensity with the A.O.A.C. method are greatest when the final pH of

the colored system is between 4.0 and 4.7, and recommends the use of sodium succinate buffer in preference to sodium sulfite as recommended in the official method.

After examining the visual colorimetric methods for the determination of phosphates recorded in the literature it was decided to investigate the method proposed by Stoloff (25). This method was chosen because the reagents used in this determination appeared to be of a more stable nature than those proposed for other methods. The method proposed by Stolloff has been changed in order to eliminate the interference of small quantities of fluoride ion.

Preliminary Experimental Work

A Beckman Model B, photoelectric self-recording spectrophotometer was used to obtain transmission curves for solutions of molybdenum blue of different hydrogen ion concentration. A description of the instrument is given in Gibb: "Optical Methods of Chemical Analysis". The optical density which is given by the expression, $d = \log 1/T$ where:

$$d = \text{optical density}$$
$$T = \text{transmittance} = \frac{I_t}{I_o}$$

is read directly from the instrument.

The transmission data for the solutions enumerated in Table XI were obtained. The pH of these same solutions was obtained using a Beckmann Glass Electrode pH meter. The color of each of the solutions was observed visually. The total volume of solution prepared, in each case, was 100 ml.

Table XI

The pH and Visual Color of the Transmission Solutions.

| Solution No. | p.p.m. $PO_4^{---}$ | ml. of Buffer | ml. of molybdate | ml. of hydroquinone | pH | Color |
|---|---|---|---|---|---|---|
| 1 | 2.5 | 8 | 8 | 8 | 4.9 | blue |
| 2 | 2.5 | 6 | 8 | 8 | 4.6 | blue |
| 3 | 2.5 | 5 | 8 | 8 | 4.3 | blue |
| 4 | 2.5 | 4 | 8 | 8 | 4.0 | blue |
| 5 | 2.5 | 2 | 8 | 8 | 2.3 | green |
| 6 | 2.5 | 0 | 8 | 8 | 2.2 | green |

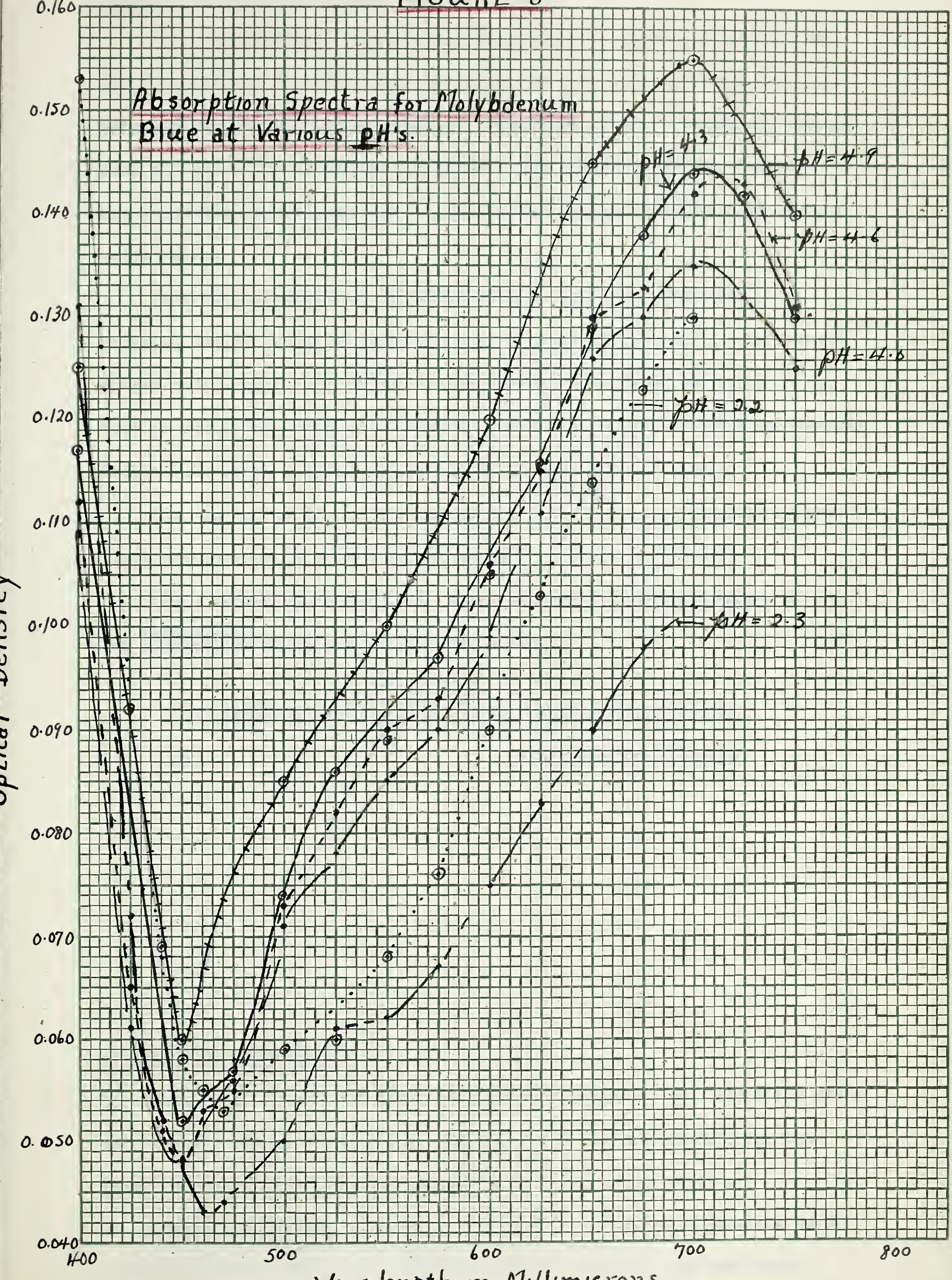
The transmission data obtained from the above solutions are given in table XII. The curves obtained by plotting optical density against the wavelength in millimicrons are shown in Fig.8.

Table XII

The Transmission Data for Molybdenum Blue.
Optical Densities.

| Wave length Millimicrons | pH 4.9 | pH 4.6 | pH 4.3 | pH 4.0 | pH 2.3 | pH 2.2 |
|---|---|---|---|---|---|---|
| 400 | 0.125 | 0.112 | 0.117 | 0.109 | 0.131 | 0.153 |
| 425 | | 0.065 | 0.065 | 0.061 | 0.072 | 0.092 |
| 440 | | 0.051 | | | 0.052 | 0.069 |
| 450 | 0.060 | 0.048 | 0.052 | 0.048 | 0.048 | 0.058 |
| 460 | | 0.053 | | | 0.043 | 0.055 |
| 465 | | 0.053 | | | | |
| 470 | | 0.051 | | | 0.044 | 0.053 |
| 475 | | 0.055 | 0.057 | 0.056 | | 0.053 |
| 500 | 0.085 | 0.073 | 0.074 | 0.071 | 0.050 | 0.059 |
| 525 | | 0.082 | 0.086 | 0.078 | 0.061 | 0.060 |
| 550 | 0.100 | 0.090 | 0.089 | 0.085 | 0.062 | 0.068 |
| 575 | | 0.093 | 0.097 | 0.090 | 0.067 | 0.076 |
| 600 | 0.120 | 0.106 | 0.105 | 0.099 | 0.075 | 0.090 |
| 625 | | 0.115 | 0.116 | 0.111 | 0.083 | 0.103 |
| 650 | 0.145 | 0.130 | 0.129 | 0.126 | 0.090 | 0.114 |
| 675 | | 0.133 | 0.138 | 0.130 | 0.098 | 0.123 |
| 700 | 0.155 | 0.142 | 0.144 | 0.135 | 0.102 | 0.130 |
| 725 | | 0.143 | 0.142 | 0.132 | | |
| 750 | 0.140 | 0.131 | 0.130 | 0.125 | | |

# FIGURE 8



Absorption Spectra for Molybdenum Blue at Various pH's.
pH = 4.3
pH = 4.9
pH = 4.6
pH = 4.0
pH = 2.2
pH = 2.3
0.160
0.150
0.140
0.130
0.120
0.110
0.100
0.090
0.080
0.070
0.060
0.050
0.040
400
500
600
700
800
Wave length in Millimicrons

Discussion of the Spectrophotometer Curves

From the careful examination of the curves in Fig. 8 it will be noted that the curves for the solutions of pH; 4.6, 4.3, and 4.0, are very similar for all wave lengths. Therefore it was considered desirable to use 5 ml. of buffer solution - the solution of pH, 4.3 in all remaining investigations. This is the median of this most stable color range.

From the examination of Table XIII it will be noted that the solution which is to be examined colorimetrically should be in the pH range: 4.0 - 4.6. In order to ensure that the final solution is in this pH range the original solution should be made just acid to phenolphthalien using a dilute solution of either NaOH or $H_2SO_4$.

The curves of the solutions of pH, 4.6, 4.3, 4.0, indicate that light absorption is at a minimum at 450 - 455 millimicrons.

The following, table XIII, shows the relationship between pH and ~~absorption~~ transmission maximum for molybdenum blue.

Table XIII
The Relationship between pH and Transmission Maximum.

| pH | Transmission Maximum |
|---|---|
| 4.9 | 450 |
| 4.6 | 450 |
| 4.3 | 450 |
| 4.0 | 450 |
| 2.3 | 460 |
| 2.2 | 475 |

Preparation of Reagents

1. I N $H_2SO_4$: 28 ml. of 36 N $H_2SO_4$ were diluted to one litre and cooled to room temperature. After the solution had cooled to room temperature the volume was again made up to room temperature.

2. Ammonium Molybdate Solution: 50 grams of ammonium molybdate were dissolved in one litre of IN $H_2SO_4$. After standing for two hours

the solution was filtered through glass wool in order to remove any insoluble materials.

3. Sodium Succinate Buffer: 36.5 grams of succinic acid and 24.7 grams of NaOH were dissolved in 250 ml. of distilled water. In the method outlined by Stoloff a solution of pure sodium succinate was used.

4. Hydroquinone Solution: 5 grams of hydroquinone were dissolved in one litre of distilled water and made slightly acid with one drop of concentrated sulfuric acid.

5. Standard Phosphate Solution: 1.43 grams of $KH_2PO_4$ were weighed out and dissolved in exactly one litre of distilled water. This gives a solution containing 1000 p.p.m. phosphate ion.

One hundred ml. of the above solution is diluted to one litre and 100 ml. of this solution is again diluted to one litre, giving a solution of 10 p.p.m. phosphate ion.

## Calibration and Operation of the Photoelectric Colorimeter for the Determination of Phosphate.

The Lumetron photoelectric colorimeter was used with a narrow band filter of 465 millimicrons. The sample holder was a cylindrical cell with a volume of 120 ml. and a light path of 15 cm. The procedure for determining data for the calibration curve is outlined below.

To a 100 ml. volumetric flask are added 8 ml. of ammonium molybdate solution, 8 ml. of hydrequinine solution, and 5 ml. of sodium succinate buffer and the volume is made up to 100 ml. This solution is placed in the sample holder, after standing for 1½ hours. The slide-wire is set at 100% transmission ~~transmittance~~ and the galvanometer is brought to zero by adjusting the angle of the balance photocell.

A series of standards are made up containing 2,5,10,15,20,25,30 40,50 and 60 ml. of the standard $KH_2PO_4$ solution. This gives a set of standards containing 0.2,0.5,1.0,1.5,2.0.2.5,3.0.4.0,5.0 and 6.0 p.p.m. of phosphate respectively. Each standard is made up as

follows: The required number of ml. of standard phosphate solution are measured out into a 100 ml. volumetric flask; 8 ml. of molybdate solution are added; 8 ml. of hydroquinone; 5 ml. of sodium succinate; the flask is brought to the mark with distilled water. The contents of the volumetric flask are thoroughly mixed after the addition of each reagent. Measuring pipettes are used to make all volume measurements.

The standards are allowed to stand for $1\frac{1}{2}$ hours. They are then transferred to the colorimeter and the percentage transmission for each standard is recorded. The results obtained from these standards are given in Table XIV.

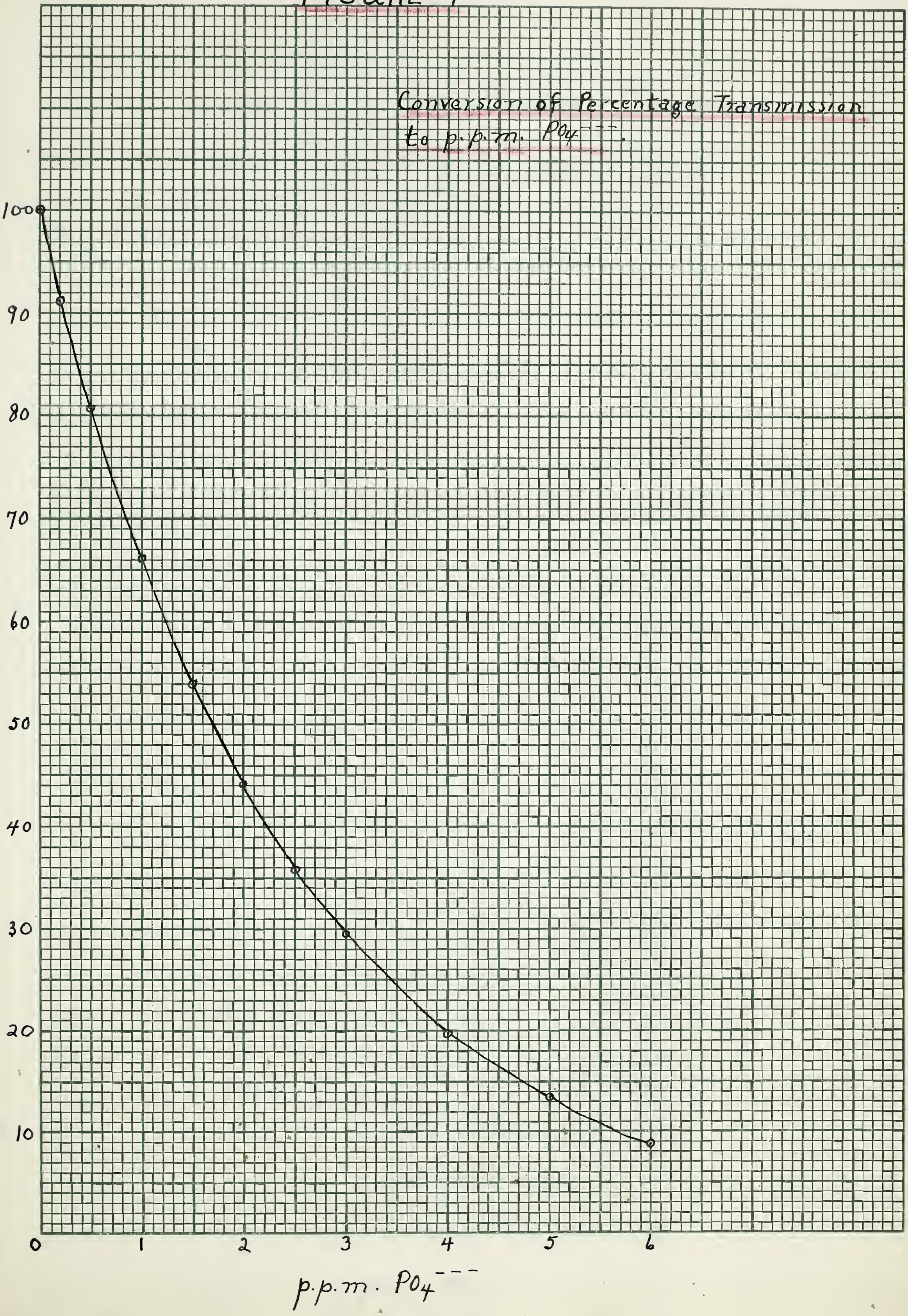
Samples of known phosphate content ranging from 0.0 p.p.m. to 6.0 p.p.m. phosphate were made up and treated according to the procedure to be detailed. Four runs were made of each concentration and the slide wire readings averaged. The averaged results are given in Table XIV. A plot of percentage transmission vs. phosphate concentration in p.p.m. gives the calibration curve for converting percentage transmission to p.p.m. phosphate is shown in fig. 9.

Table XIV
The Percentage Transmission for Phosphate Solutions.

| p.p.m.$PO_4^{---}$ | Percentage Transmission | $Log_{10}$ Percentage Transmission |
|---|---|---|
| 0.0 | 100 | 2.00 |
| 0.2 | 91.1 | 1.96 |
| 0.5 | 80.7 | 1.91 |
| 1.0 | 66.1 | 1.82 |
| 1.5 | 54.0 | 1.73 |
| 2.0 | 44.1 | 1.64 |
| 2.5 | 35.8 | 1.55 |
| 3.0 | 29.4 | 1.47 |
| 4.0 | 19.8 | 1.30 |
| 5.0 | 13.3 | 1.12 |
| 6.0 | 8.9 | 0.95 |

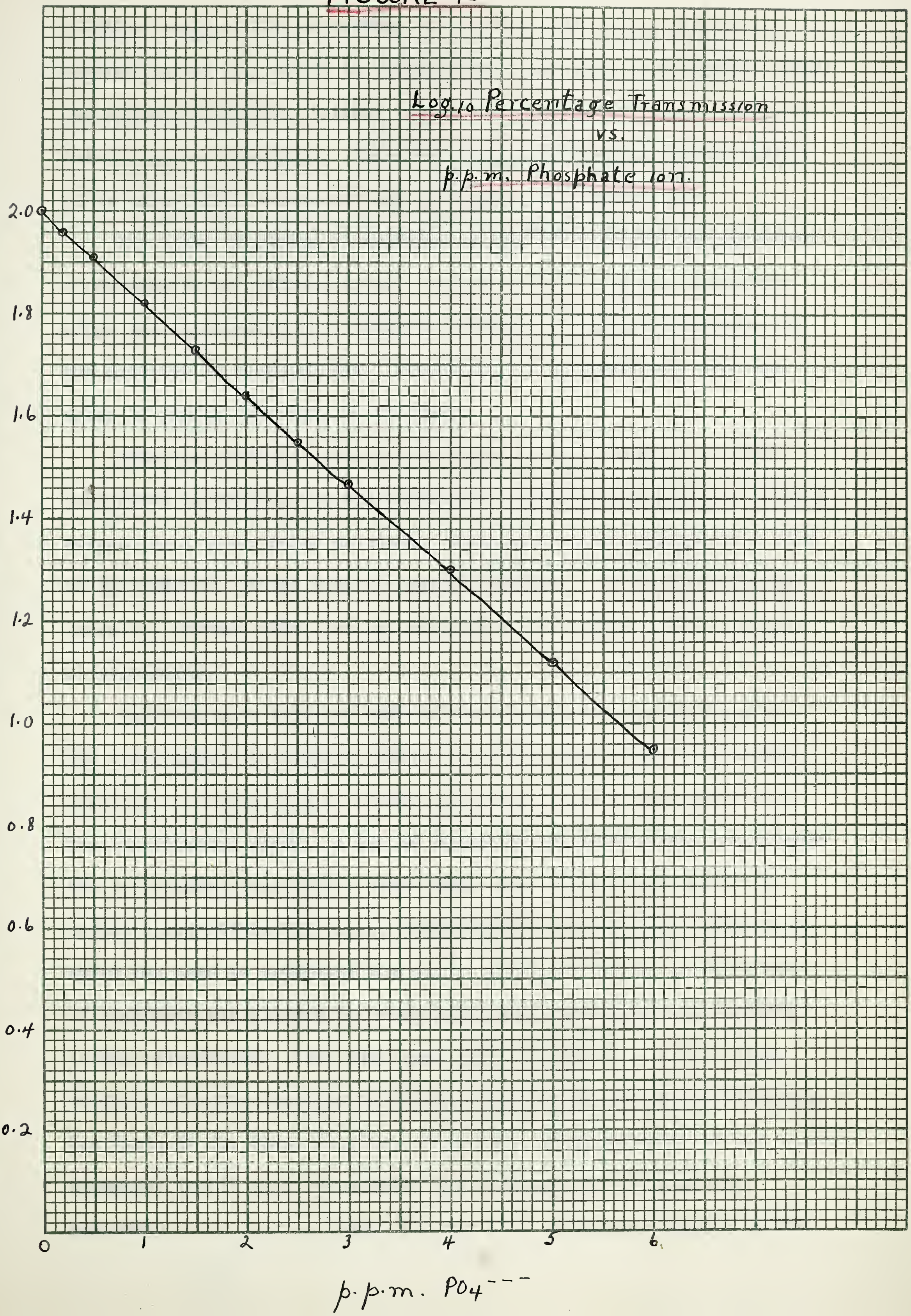
The curve obtained by plotting percentage transmission vs. the p.p.m. phosphate is hyperbolic in nature and appears to follow very closely Beer's Law. In order to confirm this a graph was constructed.

FIGURE 9



Conversion of Percentage Transmission to p.p.m. $PO_4^{---}$.
100
90
80
70
60
50
40
30
20
10
0
1
2
3
4
5
6
p.p.m. $PO_4^{---}$

FIGURE 10



Log10 Percentage Transmission
vs.
p.p.m. Phosphate ion.
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
1
2
3
4
5
6
p.p.m. PO4---

plotting $\log_{10}$ of the percentage transmission vs. p.p.m. phosphate. On examination of fig. 10 it is observed that the curve is a straight line. The system, therefore, follows Beer's law between 0.0 p.p.m. phosphate and 6.0 p.p.m. phosphate. Hence the calibration curves may be obtained by using fewer standards than the number originally used.

The order of mixing is critical in the colorimetric determination of phosphates as outlined above. If the buffer is added immediately after the addition of the molybdate solution no color is developed on the addition of hydroquinone. Therefore, it is essential, that the order of mixing outlined, be followed.

When the molybdate solution is added to the standards a yellow suspension is formed. On the addition of hydroquinone solution to this suspension a green color develops. This green color changes to a blue on the addition of the sodium succinate buffer. It is difficult to determine whether this blue solution is a true solution or a colloidal suspension.

Readings were taken on some of the standards after standing in the colorimeter for fifteen minutes from the time of the calibration readings (1½ hours) and no change was detected which was larger than the experimental error. A set of standards was made up and run through the colorimeter immediately after their formation. The results obtained agree within 0.1 p.p.m. phosphate when the 1½ hour calibration curve was used to determine the p.p.m. phosphate found. Another set of standards was allowed to develop for four hours and then compared in the colorimeter. They also gave results within 0.1 p.p.m. when compared with the readings obtained in the initial 1½ hour calibration. See Table XV for these results. Therefore, if an accuracy of 0.1 p.p.m. phosphate is all that is required the readings may be taken immediately or up to four hours from the time of the development of the

color to be measured. This accuracy is suitable for the determination of phosphate ion in conjunction with the determination of fluorides in small quantities.

Table XV.

The Effect of Time on the Transmission of Molybdenum Blue.

| p.p.m. $PO_4^{---}$ | Percentage Transmission on Immediate Mixing | p.p.m. $PO_4^{---}$ Found | Percentage Transmission after 4 hours | p.p.m. $PO_4^{---}$ Found |
|---|---|---|---|---|
| 0.0 | 100 | 0.0 | 100 | 0.0 |
| 0.2 | 89.9 | 0.2 | 89.7 | 0.2 |
| 0.5 | 80.8 | 0.5 | 81.2 | 0.5 |
| 1.0 | 65.5 | 1.0 | 66.1 | 1.0 |
| 1.5 | 53.1 | 1.5 | 55.2 | 1.4 |
| 2.0 | 42.2 | 2.1 | 45.3 | 1.9 |
| 2.5 | 34.7 | 2.5 | 37.3 | 2.4 |
| 3.0 | 28.7 | 3.0 | 30.2 | 2.9 |
| 4.0 | 18.6 | 4.1 | 21.0 | 3.8 |
| 5.0 | 12.4 | 5.1 | 14.6 | 4.8 |
| 6.0 | 8.4 | 6.0 | 9.5 | 5.8 |

The Stability of the Reagents.

The hydroquinone solution deteriorates with time. The hydroquinone solution turns brown after a month and it is therefore recommended that a new solution be prepared every week.

The sodium succinate buffer is stable and may be used for at least two months.

The molybdate solution turns blue gradually and it is recommended that a new solution of ammonium molybdate be made up every week. The blank should be checked every day and if there is any change the balance photocell should be reset so as to have the blank read 100% transmission.

The Effect of Fluorides on the Determination of Phosphates

Fluoride ions, even if present in very low concentrations, produce an interference in the molybdenum blue reaction (37), which is used extensively for determining small amounts of phosphate.

Fluorides form a complex fluoboric acid with borates. Therefore, it was decided to investigate the effect of borates on the Scott method for determining fluorides and observe whether the fluoride and phosphate effect on the indicator could be isolated from each other. Known amounts of borate were added to solutions containing a constant amounts of fluorine and their apparent fluorine content determined, by the method of Scott, using the photoelectric colorimeter. The results are given in Table XVI. The borate was added as boric acid c.p.

Twenty grams of $H_3BO_3$ were dissolved in one litre of distilled water to give a stock solution containing 19,000 p.p.m. borates.

From table XVI one may observe that borates do not interfere when added in concentrations up to 286 p.p.m. It can, therefore, be concluded that the Scott method can be used for determining fluoborate ion as well as fluoride ion.

Table XVI

The Effect of Borates on the Scott Method.

| p.p.m. $BO_3^{---}$ | p.p.m. $F^-$ added | p.p.m. $F^-$ found |
|---|---|---|
| 48 | 0.0 | 0.0 |
| 95 | 0.0 | 0.0 |
| 143 | 0.0 | 0.0 |
| 286 | 0.0 | 0.0 |
| 48 | 1.4 | 1.4 |
| 95 | 1.4 | 1.4 |
| 143 | 1.4 | 1.5 |
| 286 | 1.4 | 1.4 |

Fluoride removal is unnecessary when boric acid is added to the fluoride containing aliquot before the phosphate determination is made. The boric acid forms fluoborate ion and hence prevents the interference by fluoride ion (16).

A few solutions of constant phosphate content and varying fluoride content were developed and run through the colorimeter. On examining table XVII one will observe fluorides produce a positive interference on the determination of phosphates. Even very low concentrations cause some interference.

Tabb XVII

Fluoride Interference in Phosphate Determination

| p.p.m. $F^-$ | p.p.m. $PO_4^{---}$ | Percentage Transmission | p.p.m. $PO_4^{---}$ found |
|---|---|---|---|
| 0.0 | 1.0 | 65.7 | 1.0 |
| 0.2 | 1.0 | 65.6 | 1.0 |
| 0.5 | 1.0 | 65.7 | 1.0 |
| 1.0 | 1.0 | 64.3 | 1.0 |
| 2.0 | 1.0 | 61.2 | 1.2 |
| 3.0 | 1.0 | 56.8 | 1.3 |
| 0.0 | 2.5 | 35.2 | 2.5 |
| 0.5 | 2.5 | 33.7 | 2.6 |
| 1.0 | 2.5 | 33.2 | 2.7 |
| 1.5 | 2.5 | 32.5 | 2.7 |
| 2.0 | 2.5 | 30.5 | 2.8 |

## The Effect of Borates on the Determination of Phosphates as Applied to the photoelectric Colorimeter

Boric acid forms fluoborate ion with fluoride ion and therefore prevents the interference by fluoride ion (16).

A few solutions of constant phosphate concentration and varying borate content were developed and run through the photoelectric colorimeter. It was found that borates do not interfere (see table XVIII).

Table XVIII

The Effect of Borates on Phosphate Determination.

| p.p.m. $BO_3^{---}$ | p.p.m. $PO_4^{---}$ | Percentage Transmission ~~Scale Reading~~ | p.p.m.$PO_4^{---}$ found |
|---|---|---|---|
| 98 | 2.5 | 35.0 | 2.5 |
| 195 | 2.5 | 35.1 | 2.5 |
| 390 | 2.5 | 35.4 | 2.5 |

The Elimination of the Interference of $F^-$ on the Colorimetric Determination of $PO_4^{---}$

In order to determine whether or not boric acid is efficient in eliminating the interference of fluorides in the determination of phosphates a set of standards was ~~were~~ made up as detailed in the method outlined for obtaining the calibration curve with the exception that a known amount of fluoride and boric acid were added before any of the color forming reagents were added to the phosphate solution. It is essential that the boric acid be added to the sample orstandard before the addition of the color forming reagents - molybdate solution, hydroquinone solution, and the sodium succinate buffer.

The fluoride ion was added as sodium fluoride and the borate was added as boric acid. The same solutions of boric acid and sodium fluoride, as already described, were used. Two millilitres of the boric acid (20 gm. per litre) solution is equivalent to 390 p.p.m. of boric acid.

The following is a tabulation of the results obtained when 1.0 p.p.m. of fluoride are present:

Table XIX

Borates in Eliminating Fluoride Interference

| p.p.m. $PO_4^{---}$ added | p.p.m. $BO_3^{---}$ | p.p.m. $F^-$ | Percentage Transmission | p.p.m. $PO_4^{---}$ found |
|---|---|---|---|---|
| 0.2 | 390 | 1.0 | 91.6 | 0.2 |
| 0.5 | 390 | 1.0 | 81.7 | 0.5 |
| 1.0 | 390 | 1.0 | 66.9 | 1.0 |
| 1.5 | 390 | 1.0 | 54.4 | 1.5 |
| 2.0 | 390 | 1.0 | 44.7 | 2.0 |
| 2.5 | 390 | 1.0 | 36.3 | 2.5 |
| 3.0 | 390 | 1.0 | 29.8 | 3.0 |
| 4.0 | 390 | 1.0 | 20.1 | 4.0 |
| 5.0 | 390 | 1.0 | 14.0 | 4.9 |
| 6.0 | 390 | 1.0 | 9.1 | 5.9 |

The p.p.m. $PO_4^{---}$ found is obtained from the calibration curve (figure 9).

In order to determine whether this amount of borate was sufficient to tie up the maximum amount of fluoride that can be determined by the Scott modification without dilution the following solutions were compared in the colorimeter.

Table XX

| p.p.m. $PO_4^{---}$ added | p.p.m. $BO_3^{---}$ | p.p.m. $F^-$ | Percentage Transmission | p.p.m. $PO_4^{---}$ found |
|---|---|---|---|---|
| 0.2 | 390 | 1.4 | 91.7 | 0.2 |
| 1.0 | 390 | 1.4 | 66.5 | 1.0 |
| 2.5 | 390 | 1.4 | 36.5 | 2.4 |
| 5.0 | 390 | 1.4 | 13.8 | 5.0 |

Therefore, it may be observed that 390 p.p.m. of borate is sufficient to tie up the maximum amount of fluoride that can be determined by the Scott modification for determining fluorides.

## Conclusions and Results.

1. The method proposed by Stoloff for the determination of phosphates has been found applicable to a photoelectric colorimeter. Phosphates may be determined in the range 0.0 to 6.0 p.p.m. by this method when a 150 mm. absorption cell is used. When phosphates are present in greater quantities than this the sample will have to be diluted.
2. The method is accurate to 0.1 p.p.m. of phosphate ion.
3. Fluorides, when present even in very small quantities interfere with this method of phosphate determination.
4. The interference of fluorides may be eliminated by the addition of boric acid to the sample before any of the color forming reagents are added.
5. In the development of the standards and samples the order of addition of the reagents is critical. They are to be added as follows:
   1. molybdate solution.
   2. hydroquinone reagent
   3. sodium succinate buffer.

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